Triterpenes and Steroidal Compounds from *Napoleona imperialis* Seed: Spectroscopic Approach and Biological Activities Against Some Clinical Isolates

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Abstract

This study seeks to isolate, characterize and identify the bioactive compounds from Napoleona imperialis seed and its biological activities against some clinical pathogens. The extraction method employed was cold maceration whereby dried pulverized material (1 kg) was introduced into a 2.5 L Winchester bottle and hexane (1000mL) for 48 hours and the extract was intermittently shaken and was filtered into a clean one litter glass jar, and evaporated to dryness. The same procedure was done for ethyl acetate and methanol successively. Using standardized techniques, wagner reagent, mayer's reagent, dragendoff reagent and chemical tests such as froth, lead sub-acetate, ferric chloride, sodium hydroxide and salkowski tests were performed on Napoleona imperialis seed extract in this work to identify secondary metabolites such as alkaloid, flavonoid, steroid, phenol, tannins and saponins which accounts for its medicinal value. In Column chromatography techniques, the plant extracts of n-hexane, ethyl acetate and methanol were dried in sterile stainless steel plates and weighed using a weighing balance. The plant extract sample (2.5 g) of each extract was absorbed unto silica gel (2.0 g) and dried till a free flowing powder was obtained. This was done in order to get a pure and refined isolates. The thin layer chromatography (TLC) technique was used to the concentrated fractions that were obtained from the column chromatography. After careful application, a spot of each fraction was placed on a TLC plate that had been coated with silica. To determine the molecular structure of the compounds, a Pasteur pipette was used to dissolve the cleaned samples in chloroform (CDCl3) before they were transferred to clean NMR tubes and analyzed using nuclear magnetic resonance (NMR). To ascertain the anti-microbial activity, selected organisms (Bacteria isolates) were examined in the study. The organism was maintained on nutrient Broth for 24 hours. For the purpose of standardising the test bacterium, a sterile wire loop was used to select three to five pure cultures of the test microorganism. These cultures were then emulsified in three to four millilitres of sterile physiological saline. Modified disc diffusion techniques were used in order to assess the antibacterial properties of the extracts in relation to the bacteria that were being tested. The efficiency of the medicine may be explained by the presence of secondary metabolites, which were detected during the phytochemical screening of the Napoleona imperialis seed extract. These secondary metabolites include alkaloids, flavonoids, steroids, phenol, tannins, and saponins.

These phytochemicals were quantified by GC-FID. The Phytochemical screening showed epihedrine had the greatest value (45.066ppm) while kaempferol had the lowest content (0.386ppm). The antimicrobial analysis showed that the extract of Napoleona imperialis seed has potential antimicrobial activities. The growth of these organisms has been shown to be strongly inhibited by the extract of Napoleona imperialis seed, with a mean value between 13.00+ 1.00mm and 19.83+ 1.74mm. All of the isolates were sensitive to Napoleona imperialis seed extract because of the various phytochemicals contained in the sample. These features are due to the bioactive substances contained in the plant, stigmaterol, stigmaterol, and lupeol acetate. This study has justified that Napoleona imperialis seed are inestimable source of phytochemicals and the bioactive components such as sitosterol, stigmaterol and lupeol acetate, making it suitable for human consumption therefore consumers may not be at danger for any health issues.

Introduction

An evergreen non-timber plant known as Napoleona imperialis may be found in a broad variety of locations over the majority of the tropical wet zones in West Africa (Koppel, 1990). These locations include marginal fields, secondary bushes, and fallen bush areas. According to Dalziel (1955) and Keay et al. (1964), the plant, along with the cannon ball tree (corrupita guianensis), belongs to the family of lecythidaceae and may be found throughout the majority of Nigeria**.** In Nigeria's Ikwuano dialect of the Igbo language, the plant is often referred to as "Utum" (Ukpabi and Ukpabi, 2006). However, some botanists feel *Napoleona imperialis* deserves its own family, the Napoleonaceae, while others say it belongs in the Barringtoniaceae. The tree has a dense, rounded crown, dark green foliage, and is around 14.5 meters high and 10 meters in diameter. The gray to light brown tint of the bark darkens with exposure. The common stem is strong and typically rounded at the tip, and the leaflets are big and elliptic. The flowering period lasts from January to March, and the flowers, which are normally between 0.7 and 1.10 mm long and yellowish white in color, are tightly grouped on a sturdy central stem. In April, it yields a lot of globular fruits. Fruits are fleshy protrusions that are directly attached to the main stems and limbs of the trees. Despite being one of the less well-known species, Dalziel (1955) and Irvine (1961) have discussed some of *Napoleona imperialis'* economic relevance. The fruit's sweet pulp can be used as a dessert, the roots can be used as a cure, and the twigs can be used as regular chewing sticks. Different plant components are utilized for various reasons in the region, according to the studies of Osei-owusu (1981) and Okafor & Fernades (1987), including mulching and fodder (leaves and twigs), as well as firewood, chewing sticks, and ethnomedicine (stem and root). Man consumes the juice from the fruits and pods, but discards the seeds. The seeds could serve as an alternate feed ingredient for the production of cattle because they have very little appeal as human food or any industrial usage. According to a study by Uchegbu et al. (2000), the seeds of *Napoleona imperialis* contain 90.5 g of crude protein per kilogram when they are ripe and dried. Iheukwumere & Okoli (2002) found that feeding weaner rabbits raw, dry seed had negative effects on performance, hematological, and serum criteria at a dietary inclusion of 15%, however the rabbit did not have any significant influence on the same parameters at a dietary inclusion of 5%.

Uncertainty surrounds the chemical composition of seeds from *Napoleona imperialis*. A concise summary of the chemical composition of the leaf, bark, and roots was published by Ogbonnaya (1983). Additionally, Dalziel and Irvine (1955, 1961) previously discussed the possible presence of saponin in *Napoleona imperialis* seeds. The leaves can harm the digestive mucosa and produce hemolytic alterations in blood. They also have a characteristically bitter flavor and foaming qualities. Radostits et al. (1997), Macdonald et al. (1998), and Kumar & Dimello (1995). South eastern Nigeria is a frequent location for *Napoleona imperialis*. According to botany, a fruit is an auxiliary and ripened ovary that is part of a flower. A fruit is typically understood to be a fleshy, tree-specific product that is high in sugar and acidity and has a distinct flavor when mature. Many vegetables, including beans, squash, tomatoes, etc., are actually fruits. Fruits like pears, apples, plums, and peaches are eaten largely for their distinctive flavor, delicate texture, and high nutritional value in terms of vitamins and minerals.

Fruits are a significant source of pro-vitamin A, carotenoids, ascorbic acids, several minerals, as well as fiber and carbohydrates that are easily digested. Fruits come in a variety of colors, textures, and flavors, some of which are best distributed fresh on the market while others are suitable for processing. The availability of fresh fruits has increased as a result of the advancement and improvement of transportation, packing, canning, and freezing technologies. Previously, fresh fruit supplies were constrained by their seasonal nature. Fruit is frozen and canned as soon as it is harvested to help maintain the best quality and nutritional content in the final product.

Materials and Methods.

Sample collection and preparation

Seed of *Napoleonaca imperialis* were obtained in April, 2022 from Bunu Tai, Tai Local Government Area, Rivers State, Nigeria and authenticated at Reference Laboratory Section of Conig-Simonne Laboratories, Awka, Anambra State, Nigeria. The sample was washed with distilled water, air dried for 3 weeks and grinded to powder using a grinding machine. Thereafter, it was stored in a plastic container and taken to the Laboratory for analysis

Extraction procedure (Cold Maceration)

The dried pulverized material (1 kg) was introduced into a 2.5 L Winchester bottle and hexane (1000mL) for 48 hours with intermittent shaking the extract was filtered into a clean one litter glass jar, and evaporated to dryness. The same procedure was done for ethyl acetate and methanol successively. The extraction method employed was cold maceration.

Phytochemical screening

Chemical tests were carried out on *Napoleonaca imperialis* extract to identify secondary metabolites such as alkaloids, flavonoids, steroids, phenolic, tannins, and saponins using standard procedures as demonstrated by (Aiyelaagbe & Osamudiamen, 2009; Edeoga et al. 2005)

Preparation of sample for GC analysis

After dissolving one millilitre of filtered residue in twenty millilitres of chloroform, the mixture was placed into one hundred millilitres of volumetric flask and diluted to the mark. After the majority of the chloroform was evaporated at ambient temperature, one millilitre of the reagent consisting of 10 vol% benzene and 20 vol% methanol} was added. The mixture was then sealed and heated in a water bath at a temperature of 400 degrees Celsius for thirty minutes. After that, the organic sample was extracted using hexane and water, and the final combination of the reagent, which consisted of hexane and water, was in the proportion of 1:1:1 (that is, one millilitre of hexane and one millilitre of water were added to the reaction mixture). For a period of two minutes, the liquid was forcefully agitated by hand until an emulsion that was stable was acquired. When the organic layer was removed, half of the top hexane phase was transferred to a tiny test tube for injection (Nna, 2023) after which the organic layer was removed.

Melting Points Determination of Isolated Samples

The SMPI Stuart Scientific melting point device was used to measure the melting point of each pure chemical.

Column Chromatography

The n-hexane and ethyl acetate extracts were dried in sterile stainless steel plates and weighed using a weighing balance. The plant extract sample (2.5 g) of each extract was absorbed unto silica gel (2.0 g) and dried till a free flowing powder was obtained. The column was packed with a slurry of silica gel (50 g) prepared with hexane and ethyl acetate solvent mixture mixed in the ration 180 mL: 20 mL for each extract of n-hexane, ethyl acetate and methanol. A plug of cotton wool was introduced into the column to prevent silica gel from escaping into the collection vials and also provide an even base line for the silica gel slurry. The slurry would be even introduced in a one smooth flow and the solvent drain off just to the top of the column bed in each of the column. The columns for n-hexane, ethyl acetate and methanol were gently tapped with a rubber hose to release trapped air bubbles in order to forestall column cracking. A dried free flowing mixture of plant extract adsorbed unto silica gel for plant extracts of n-hexane, ethyl acetate and methanol were introduced unto their various silica gel beds. A solvent mixture of (hexane: ethyl acetate) in the ratio 180 mL: 20 mL were used to wash down sides of the column and also fill it up. The plant extracts of n-hexane, ethyl acetate and methanol were allowed to settle as different band separations were formed in the various columns. Solvent mixture of hexane and ethyl acetate (95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) was added to begin elution. Fractions were collected in vials at different intervals for columns of n-Hexane, ethyl acetate and methanol (Igoli et al., 2011, Nna and Okwelle.,2022).

Thin Layer Chromatography (TLC) Analysis

The thin layer chromatography (TLC) technique was used to the concentrated fractions that were obtained from the column chromatography.After careful application, a spot of each fraction was placed on a TLC plate that had been coated with silica. Following a period of about five minutes, the plate was submerged in an appropriate solvent, which enabled the chemical that was present in the area to rise higher due to the capillary attraction. Three different solvent systems were used:

9:1 volume/volume hexane in ethyl acetate was utilised for fractions 1-20, 8:2 was utilised for fractions 21-50, and 7:3 was utilised for fractions 51-80. After that, the plate was set aside to dry after being taken from the solvent. After spraying the plates with a mixture of 20% sulfuric acid and methanol, they were then heated and blackened. The formula that was used to calculate the retention factor Rf values of each and every place is as follows:

$$R_f = \frac{\textbf{\textit{Distance travelled by the spot}}}{\textbf{\textit{distance travelled by the solvent}}}$$

Fractions with similar Rf values were combined together and labeled as NIS-21 and NIS-23. These fractions were subjected to spectroscopic analysis and spectra values obtained were compared with literature values of compounds isolated, characterized and identified from the plant under investigation.

Nuclear Magnetic Resonance (NMR) Spectroscopic Analysis

To determine the molecular structure of the compounds, a Pasteur pipette was used to dissolve the cleaned samples in chloroform (CDCl3) before they were transferred to clean NMR tubes and analyzed using nuclear magnetic resonance (NMR). Bruker Avance 3 spectrometer was used to record the NMR studies at 400 MHz (1H) and 376 MHz (13C). Chemical shifts in ppm were normalized to CDCl3 (Deuterochloroform) at a value of 7.26 since all spectra were collected in this solvent. Splitting patterns were denoted as singlets (s), doublets (d), doublet of doublets (dd), triplets (t), and multiplets (m). Coupling constants (J) were expressed in Hertz (Hz) (m).

Antimicrobial Activity of Extracts against some Selected Test Bacteria

The organisms used in this present study were obtained from the Department of Human Pathology, University of Port Harcourt Teaching Hospital, Port Harcourt and maintained on Nutrient Broth for 24 hours.

Standardization of Test Bacteria

For the purpose of standardising the test bacterium, a sterile wire loop was used to select three to five pure cultures of the test microorganism. These cultures were then emulsified in three to four millilitres of sterile physiological saline. At a wavelength of 540 nm, the turbidity reading of the 0.5 McFarland standard was recorded as absorbance in a Spectrophotometer. At the same time, the turbidities of the test organisms were corrected to match the absorbance of the 0.5 McFarland standard at the same wavelength by applying physiological saline. Please take note that 0.5 McFarland contains 1.5x108 colony-forming units per millilitre (Nna et al., 2019).

Antimicrobial Susceptibility Test

According to Agu et al. (2013) and Adindu et al. (2016), modified disc diffusion techniques were used in order to assess the antibacterial properties of the extracts against the bacteria that were being tested. The Mueller-Hinton plates were cultivated using the pour plate technique, and exactly 25 microliters of a 0.5 McFarland standardised suspension of test bacteria (1.5 x 108 colony-forming units per millilitre) were used. For the purpose of impregnating the 6mm filter paper discs, exactly 50 µl of the extracts were used. These discs were then put on two different sections of the agar plate. The sizes of the inhibition zones on each of the plates were measured and recorded in millimetres throughout the experiment. It was necessary to do each experiment three times.

Negative controls were established by using sterile physiological saline, whereas positive controls were established by employing Ciprofloxacin at a concentration of 50 µg/ml.Minimum Inhibitory Concentration (MIC) is determined by this process.

Determination of Minimum Inhibitory Concentration (MIC)

The approach that was utilised for this investigation was modified from the one that was used by Pallota et al. (2007) by Agu et al. (2013). The broth dilution procedure was used in order to ascertain the Minimum Inhibitory Concentration (MIC that was determined. A dilution factor of 10-2 was used for fungi, while a dilution factor of 0.5 McFarland adjusted cultures were used for bacteria and yeast. Nutrient Broth and Sabouraud Dextrose Broth, which contained 20%, 40%, 60%, and 80% of the extracts, were inoculated with known quantities of the bacterial and fungal inocula. In addition, controls, both positive and negative, were established. In a metabolic rotary shaker with a speed of 220 revolutions per minute, the tubes were incubated at room temperature for a period of 24 hours for bacteria and for a period of 48 hours for fungus. In the next step, the test tubes that had been incubated were subcultured onto sterile plates that had been newly produced. These plates were then incubated for a period of 24 hours for bacteria and 48 hours for fungi. The plates were counted at the conclusion of the incubation time, and the total microbial count was reported in colony-forming units per millilitre. The minimum inhibitory concentration (MIC) was determined to be the plate that had the lowest count. According to Nna and Ejiofor (2023), the minimum inhibitory concentration (MIC) is defined as the lowest concentration that is necessary to successfully halt the growth of microorganisms after either 24 or 48 hours of incubation.

Results and Discussion

Table 1. Qualitative Phytochemical screening of *Napoleona imperialis* seed

S/N	Phytochemical components	Remark
1	Alkaloids	+
2	Flavonoid	+
3	Steroid	+
4	Phenol	+
5	Tannins	+
6	Saponins	+

Table 2. Quantitative Phytochemical screening *Napoleona imperialis* seed

S/N	Phytochemical	Composition ppm
1	Kaempferol	0.386
2	Catechin	0.873

3	Steroids	3.093
4	Aphyllidine	5.483
5	Anthocyanin	7.873
6	Naringenin	9.35
7	Dihydrocyticine	14.036
8	Ammodendrine	19.973
9	Tannins	20.616
10	Cyanogenic glycoside	23.363
11	Flavonones	25.96
12	Flavones	29.326
13	Ribalinidine	31.653
14	Spartein	34.15
15	Phytate	36.773
16	Oxalate	38.64
17	Sapogenin	43.536
18	Epihedrine	45.066

Table 3: Proton NMR Data for NIS-21

Position	**Experimental**	**Jonathan & Okieimen (2020)**	**Experimental**	**Pateh et al., (2009)**
1				1.47
2				1.56
3	3.51 (m)	3.51 (m)	3.51 (m)	3.52
4				2.28
5				-
6	5.35 (dd, 4.9 2.70)	5.35 (dd, 4.9 2.70)	5.35 (dd, 4.9 2.70)	5.34
7	2.00 (ttd, 11.7, 8.7, 4.51)	2.00 (ttd, 11.7, 8.7, 4.51)	2.00 (ttd, 11.7, 8.7, 4.51)	2.03
8				1.67
9				1.48

10				
11				1.52
12				1.49
13				-
14				1.50
15				1.60
16				1.84
17	1.01 (s, 3H)	1.01 (s, 3H)	1.01 (s, 3H)	1.49
18	0.80 (s, 3H)	0.80 (s, 3H)	0.80 (s, 3H)	0.68
19				1.02
20				0.94
21	1.84 (m)	1.84 (m)		0.88
22	5.01 (dd, 15.17, 8.60)	5.01 (dd, 15.17, 8.60)	1.04	1.04
23	5.15 (m)	5.15 (m)	1.52	1.50
24	2.29 (m)	2.29 (m)		1.65
25				0.83
26	0.82 (s)	0.82 (s)	0.82 (s)	0.85
27	0.85 (s, 3H)	0.85 (s, 3H)	0.85 (s, 3H)	1.04
28				0.88
29	0.68	0.68	0.68	2.00
30				1.52

Table 4: Proton NMR Data for NIS-23

Position	**Experimental**	**Ipav et al., (2022)**
1		
2		1.64
3	4.49 (m)	4.59 (m)
4		0.73
5		1.42
6		
7		
8		
9		

10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23	0.79 (s)	0.79
24	0.83	0.83
25	0.85 (s)	0.85
26	0.96	0.97
27	0.98 (d, 0.85)	0.99
28	1.03 (s)	1.04
29a	4.68 (d, 2.53)	4.70
29b	4.57 (dd, 2.61)	4.59
30	1.68 (dd, 1.45, 0.72)	1.71
1’	-	-
2’	2.04 (s)	2.05

Table 5: Morphological and Biochemical Identifications of the Various Isolates of the Test Bacteria.

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Ci
PA	Circular	Smooth	Whitish	Entire	Convex	Translucent	- Rod	+	+	-	-	-	+
ST	Circular	Smooth	Greyish/ white	Lobate	Low convex	Translucent	-Rod	+	+	-	+	-	-
SA	Circular	Smooth	Yellowish	Entire	Raised	Opaque	+ cocci	+	+	-	+	-	-
EC	Circular	Smooth	Whitish	Entire	Convex	Translucent	-Rod	+	+	+	+	-	-
EF	Circular	Smooth	cream	Entire	convex	Opaque	+coccus	-	-	-	-	+	-

Key:

Gram: Gram reaction
Cat: Catalase test
Mot: Motility test
Ind: Indole test
MR: Methyl-red test

VP: Voges-Proskauer test
Cit: Citrate Utilization test
Sugar Fermentation Tests:
Lac: Lactose Fermentation
Glu: Glucose Fermentation
Suc: Sucrose Fermentation
Fru: Fructose Fermentation
Mal: Maltose Fermentation
Oxi : Oxidase
Ure: Urease

Table 6: Preliminary antimicrobial susceptibility screening of *Napoleona imperialis* Seed

	Plant Extract				**+ve ctrl**				**-ve ctrl**			
Test Bacteria	x	y	z	Mean±SD	x	y	z	Mean±SD	x	y	z	Mean±SD
Salmonella typhi	13	12	14	13.00±1.00	27	28	28	27.67±0.58	-	-	-	-
Staphylococcus aureus	0	0	0	0.00±0.00	43	43	43	43.00±0.00	-	-	-	-
Pseudomonas aeruginosa	18	20	21.5	19.83±1.76	40	43	41	41.33±1.53	-	-	-	-
Enterococcus faecalis	15.5	16	17	16.17±0.76	45	47	47	46.33±1.15	-	-	-	-
Escherichia coli	19.5	21	19	19.83±1.74	45	45	46	45.33±0.58	-	-	-	-

Qualitative Phytochemical screening of *Napoleonaca imperialis* seed

The crude extract of *Napoleonaca imperialis* seed was subjected to preliminary phytochemical analysis, which detected the presence of alkaloids, flavonoids, steroid, phenol, tannins and saponins (Table 1). These compounds make excellent sources of ingredients for products used in both food and medicine. A natural defense mechanism against viruses, parasites, fungus, and insect herbivores, phytochemicals are used by plants to provide scent, color, and flavor, according to

Ibrahim et al. (2010). Therefore, the presence of these compounds in the seed can be credited for the *Napoleonaca imperialis* seed medicinal efficacy.

Quantitative Phytochemical screening *Napoleonaca imperialis* seed

The seed of *Napoleona imperialis* included a total of eighteen (18) phytochemical components that were identified and quantified. The results shows that Kaempferol has the lowest concentration (0.386ppm) and Epihedrine has the highest concentration (45.066ppm). The phytochemical substances that the sample included were Kaempferol (0.386ppm), Catechin (0.873ppm), Steroids (3.093ppm), Aphyllidine (5.483ppm) Anthocyannin (7.873ppm), Naringenin (9.350ppm), Dihydrocyticine (14.036ppm), Ammodendrine (19.973ppm), Tannins (20.616ppm), Cyanogenic glycoside (23.363ppm), Flavonones (25.960ppm), Flavones (29.326ppm), Ribalinidine (31.653ppm), Spartein (34.150ppm), Phytate (36.773ppm), Oxalate (38.640ppm), Sapogenin (43.536ppm), Epihedrine (45.066ppm).

Napoleona imperialis seed contain alkaloids, flavonoids, steroids, phenol, tannins, and saponins, according to a phytochemical research. In the pharmacology and treatment of humans, these phytochemicals are crucial (Yasir et al., 2010). In humans, alkaloids, for instance, have pharmacological effects that are anti-malarial, anti-cancer, anti-asthmatic, and antibacterial. According to Akinpelu et al. (2014), nutraceuticals and dietary supplements containing saponins are becoming more and more common. Saponins are also employed as a blood cholesterol reducer and an anticancer agent. Additionally, its amphipathic properties encourage protein translocation via cell membranes. The ability of glycosides to function as antibiotics is widely established. The phytoconstituents obtained in this investigation can be effectively compared with studies from earlier literature (Owolabi et al., 2010; Yasir et al., 2010). Due to their antifungal and antibacterial properties, alkaloids like aphyllidine, dihydrocyticine, ammodendrine, ribalinidine, spartein, and epihedrine protect plants from pests like insects and herbivores as well as bacteria and fungus, which is essential for plant survival (Molineux et al., 1996). Alkaloid plant-based chemicals have been used as poisons, dyes, and medicines ever since the birth of civilization. Indole alkaloids have many positive health effects, including the prevention of cancer, high blood pressure, malaria, and arrhythmia. There simply aren't enough examples to support this set of plant components' incredibly profitable relevance. Alkaloids are used as analgesics and antimalarial drugs and have similar intoxicating qualities to nicotine, morphine, caffeine, and quinine (Wink et al., 1998). Alkaloids are chemical compounds with fundamentally consistent structures and heterocyclic nitrogen atoms. The term "alkaline" refers to any base that contains nitrogen, which is how alkaloids got their name (Muller-Harvey, 1999). Typically, alkaloids taste bitter. The alkaloid quinine, which has a significant bitterness (1x10-5) at a molar concentration, is one of the substances that are known to be bitter (Mishra, 1989). Since there are many alkaloids and they come in a variety of molecular forms, it is difficult to logically classify them. Each molecule can be classified into a family according to the type of heterocyclic ring system it possesses (Krishnan et al., 1983). Following is a list of the several classes of alkaloids, organized by the heterocyclic ring system that each one of them possesses. Tetrahydropyrroles, or pyrrolidine rings, make up their structure. alkaloids of pirolidine. For instance, the chemical hypgrine can be found in the leaves of Erythroxylum species and Leonotis species. The pyridine alkaloids are composed of hexahydropyridine, a substance with a piperidine ring structure. Take the substances coniine,

piperine, and isope-lletierine as examples. Two pyridines make up the alkaloids.These have the heterocyclic ring system of pyrrolidi-nepyridine. For instance, tobacco (Nicotianatabaccum) contains the nicotine alkaloid myosmine. The pyridine ring system and the piperidine ring system are connected in this group of alkaloids, also referred to as pyridine-piperidine alkaloids. Anabasis aphyllan contains the anabasine alkaloid. Quinoline alkaloids share the same fundamental heterocyclic quinoline ring structure as quinoline. For instance, the cinchona tree's bark contains quinine. These alkaloids contain isoquinoline that is organized in a heterocyclic rig structure. Numerous substances, such as heroin, morphine, codeine, and papaverine, are opiate alkaloids. Kaempferol, catechin, naringenin, anthocyanin, flavonones, and flavones are a few examples of flavonoids. Flavonoids appear to have been used for a very long time and may have been essential for the earliest successful medicinal therapies. When they spontaneously combine with sugar, many flavonoids take on conjugated forms. In addition to D-glucose and L-rhamnose, carbohydrates also contain galactose, gluco-rhamnose, arabinose, and gluco-rhamnose. More frequently than not, the glycosidic bond may be found at either position 3 or position 7. In recent times, there has been a significant amount of interest in the wide variety of pharmacological and biological effects that flavonoids have shown. According to research conducted by Shirsat et al. (2012) and Teiten et al. (2013), flavonoids possess a wide range of biological properties, some of which include the potential to be cytotoxic, anti-microbial, anti-inflammatory, and anti-tumor. On the other hand, absolutely every kind of flavonoids demonstrates an extraordinary capacity to function as potent antioxidants, which are capable of shielding the body from potentially harmful free radicals and potentially unstable oxygen species. The ROS. For this reason, the bulk of the people who belong to this group are referred to as "saponin," and they are responsible for producing stable foam in aqueous solutions such as soap. The chemical classification of saponins includes triterpenoids, steroid alkaloids, and glycosylated steroids, among other types of steroids. There are two types of steroid aglycones that are most often used: spirostan derivatives and furostan derivatives. It has been shown by Bohlmann et al. (1998) that the main triterpeneaglycone is a derivative of oleanane. There are one or more sugar moieties that make up the carbohydrate component. These sugar moieties are glycosidically coupled to a sapogenin (aglycone) and include glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid. Saponins with just one sugar molecule linked at the C-3 position are known as monodesmo sidesaponins. On the other hand, bidesmoside sidesaponins are saponins that have at least two sugars associated to them, with one attached at the C-3 position and the other attached at the C-22 position (Lasztity et al., 1998)**.** A saponin is a chemical similar to sapogenin. It is common knowledge that saponins have anti-microbial properties that aid in inhibiting the growth of mold and guarding plants against pests. Saponins, a large class of defensive compounds, are found in plants. Common names for them include phytoprotectants and phytoanticipins. They might be thought of as a component of the defense mechanisms employed by plants (Lacaille-Dubois & Wagner, 2000). Plant and plant product saponin combinations have a variety of biological effects when they are present in an animal's body. Studies have focused on the properties of saponins, including their permeability of membranes, immunostimulant activity, impact on lowering cholesterol, and anti-carcinogenic potential. It has also been demonstrated that these compounds have a major impact on the development, feed consumption, and reproduction of animals. When exposed to these physically

different compounds, molluscs and protozoans have been found to die. It hinders the absorption of vitamins and minerals by the intestines, acts as an antioxidant, inhibits protein digestion, raises blood sugar levels, and has antiviral and antifungal properties (Morreissy & Osbourn, 1999; Takechi et al., 1999).

Scientists have recently begun to pay attention to tannins because of the increased incidence of deadly diseases like AIDS and cancer. In particular, in light of the well-established biological effects of plant extracts containing tannins, the search for new lead compounds for the development of novel pharmaceuticals has gained relevance (Mueller-Harvey, 1999).
Antibacterial phytochemicals include steroids, flavones, and tannins (Sodipo et al., 1991). This is a result of their comprehension of how phytochemicals prevent bacterial development by precipitating microbial protein and denying it to the bacteria. When combined with digestive enzymes, alkaloids and saponins can hasten the breakdown of certain proteins (Abara 2003). Alkaloids and saponin, two bioactive substances, have been shown in studies to provide plants their antibacterial properties (Odugbemi, 2006). This demonstrates the *Napoleona imperialis* seed's capacity to prevent the development of particular insects as well as its potency in treating diseases like malaria and other problems of a similar nature. Several of these components were evaluated using a GC-FID (gas chromatography flame ionization detector).

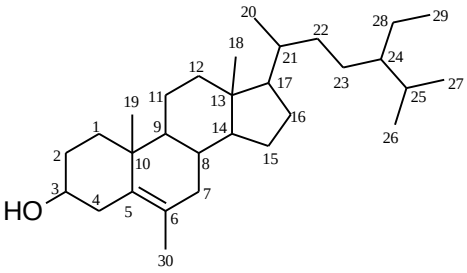
Characterisation of NIS-21 as mixture of sitosterol and stigmasterol

138-139 degrees Celsius was the melting point of the colourless needle-like crystal that was known as NIS-21. After being heated and sprayed with 20% sulfuric acid, the TLC plate of the sample displayed a pink spot. The Rf value of was calculated to be 0.50; the ratio of hexane to acetyl acetate was 9:1. The results of the test for steroids that Liberman Buchard underwent (Talukdar & Chaudhary, 2010) were likewise positive. All of these pointed to the possibility that NIS-21 is a steroid or triterpenoid. NIS-21's 1H-NMR spectra, which were obtained in CHCl3, revealed signals ranging from 5.35 ppm to 0.70 ppm in the chemical shift. There were multiplets seen at δH 5.35 ppm and δH 3.51 ppm in the NIS-21. In accordance with the findings of Shimbe et al. (2016), these signals are indicative of olefinic protons. Other indications at δH 0.69 ppm and 1.02 ppm correlate to signals that are common for angular methyl protons, as stated by Pateh et al. in 2009. When the 1H-NMR data of NIS-21 is compared with the data of previous studies, such as those by Tor-Anyiin et al. (2016), Mouffok et al. (2012), Saeidnia et al. (2014), and Arora et al. (2013), it is evident that NIS-21 is a β-sitosterol. A comparison of the 1H-NMR data of NIS-21 with reports found in the literature was presented in Table 1. According to Habib et al. (2007), stigmasterol and β-sitosterol are nearly always found in combinations with one another. It is possible for someone to have a bigger proportion of stigmasterol or β-sitosterol in the mixture. Based on the findings found in the literature, it is quite challenging to acquire Stigmasterol and β-sitosterol in highly pure forms. The primary difference between the two compounds lies in the existence of a double bond between carbon-22 and carbon-23 in the case of Stigmasterol, but in the case of β-sitosterol, there is only a single link between carbon-22 and carbon-23. Furthermore, Stigmasterol and β-sitosterol have similar R_f value of 0.55. (Kamboj &Saluja, 2011; Pateh et al., 2009).

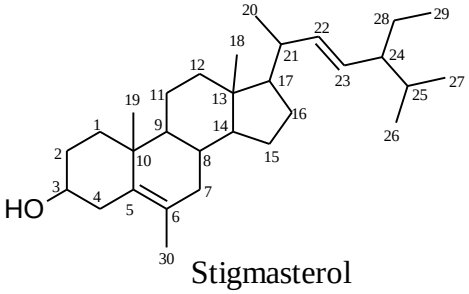
Characterization of NIS-23 as lupeol acetate

The ^{1}HNMR spectrum of NIS-23 showed presence of 8 methyl proton signals at δ_H 0.79, 0.83, 0.85, 0.96, 0.98, 1.03, 1.68 and 2.04 ppm, all of which were integrated to 3 protons. These signals are attributable to a triterpene. The signals at δ_H 4.68 and 4.57 are characteristic of germinal olefinic protons at C-29a and C-29b of lupeol. Furthermore, the coupling constants (4.57; dd, 2.61) and (4.68; d, 2.53) for the two vinyl protons confirmed that they are attached to same carbon atoms. This is because, cis- and trans- vinyl protons have 10.2 and 15.9 coupling constants, respectively. Triterpene with two different proton signal attached to same carbon are likely to be a lupeol. However, there was absence of 3.5-3.7ppm signal which is common to C-3 proton of lupeol or other sterols. Rather, a multiplet peak was seen at δ_H 4.49 is an indication that the -OH at C-3 could be acylated. Similarly, the signal δ_H 2.04 ppm is common of methyl protons of acyl group. NIS-23 was thus identified as lupeol acetate based on analysis of NMR data and comparison with literature reports.

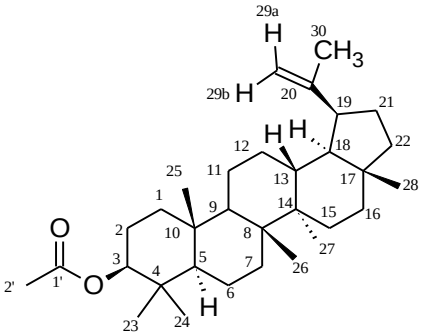
Lupeol acetate is a pentacyclic triterpene that have been isolated from several plants and other sources like propolis (Ipav et al., 2022). Pharmacological studies showed that lupeol acetate possess anti-fertility (Gupta et al., 2005), anti-inflammatory (Chen et al., 2012), anti-arthritic (Kweifio-Okai & Caroll, 1993), anti-diabetic (Fred-Jaiyesimi & Ibukunoluwa, 2016; Olanudun et al., 2018) anti-nociceptive (Chen et al., 2012), antiglycaemic and anti-venom (Chatterjee et al., 2006) properties.



Sitosterol



Stigmasterol



Lupeol acetate

Morphological and Biochemical Identifications of the Various Isolates of the Test Bacteria.

Napoleona imperialis seed antibacterial properties were demonstrated by the identification of the several test bacterial isolates utilizing morphology and biochemistry. All organisms have a spherical shape. All living creatures are fully capable of absorbing light without any reflection or transmission, with the exception of transparent *Salmonella typhi, Pseudomonas aeruginosa and Escherichia coli*, which explains why. They are all rod-shaped, gram negative bacteria, with the exception of *Enterococcus faecalis, Staphylococcus* aureus, which are gram positive cocci.

Preliminary antimicrobial screening *Napoleona imperialis* Seed

A total number of five bacteria strains were used to determine whether *Napoleona imperialis* Seed is sensitive to those five bacterial strains and to determine their antibacterial properties. *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Enterococcus faecalis, and Escherichia coli* were among these bacteria. The antibacterial activity of the various extracts was assessed using the zone of inhibition (expressed in mm) of the test microorganisms. The size of this zone depends on how well the extract prevents microbial growth. Larger zones are frequently caused by higher antibiotic concentrations. Numerous phytochemicals could be the cause of this sensitivity (Manisha & Shyamapada 2011). The growth of these organisms is significantly slowed down by the seed of the *Napoleona imperialis* extract, as shown in Table 5, with a mean value ranging from 13.00+ 1.00mm to 19.83+ 1.74mm. The average values for *Salmonella typhi and Enterococcus faecalis* were 27.67+0.58mm and 46.33+1.15mm, respectively, as reported in Table 5. *Salmonella enterica, Citrobacter murliniae, Bacillus licheniformis, Micrococcus roseus, Bacillus subtilis, and Staphylococcus aureus* are only a few examples of common infections that can lead to a wide spectrum of illnesses (Odoki et al., 2019). Because there were so many phytochemicals in the sample, all of the isolates responded to the seed extract of *Napoleona imperialis* (Manisha & Shyamapada, 2011).

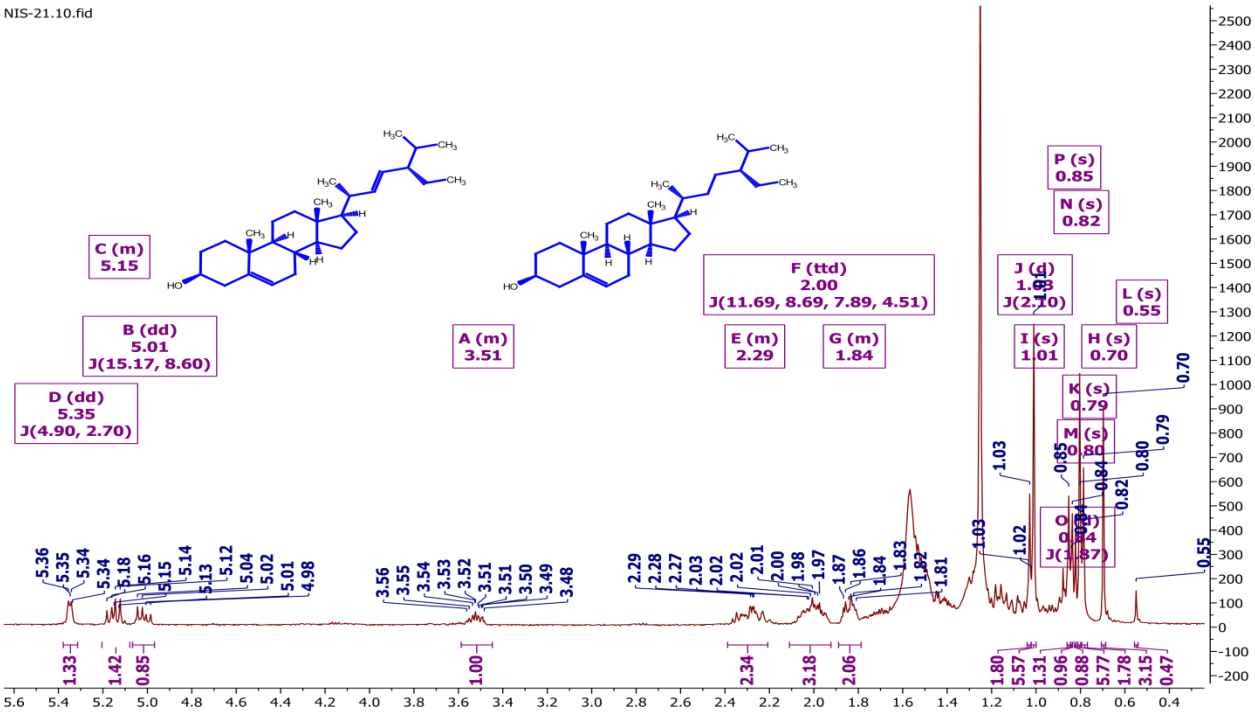


FIG 1: H-NMR SPECTRA OF STEROIDAL COMPOUNDS.

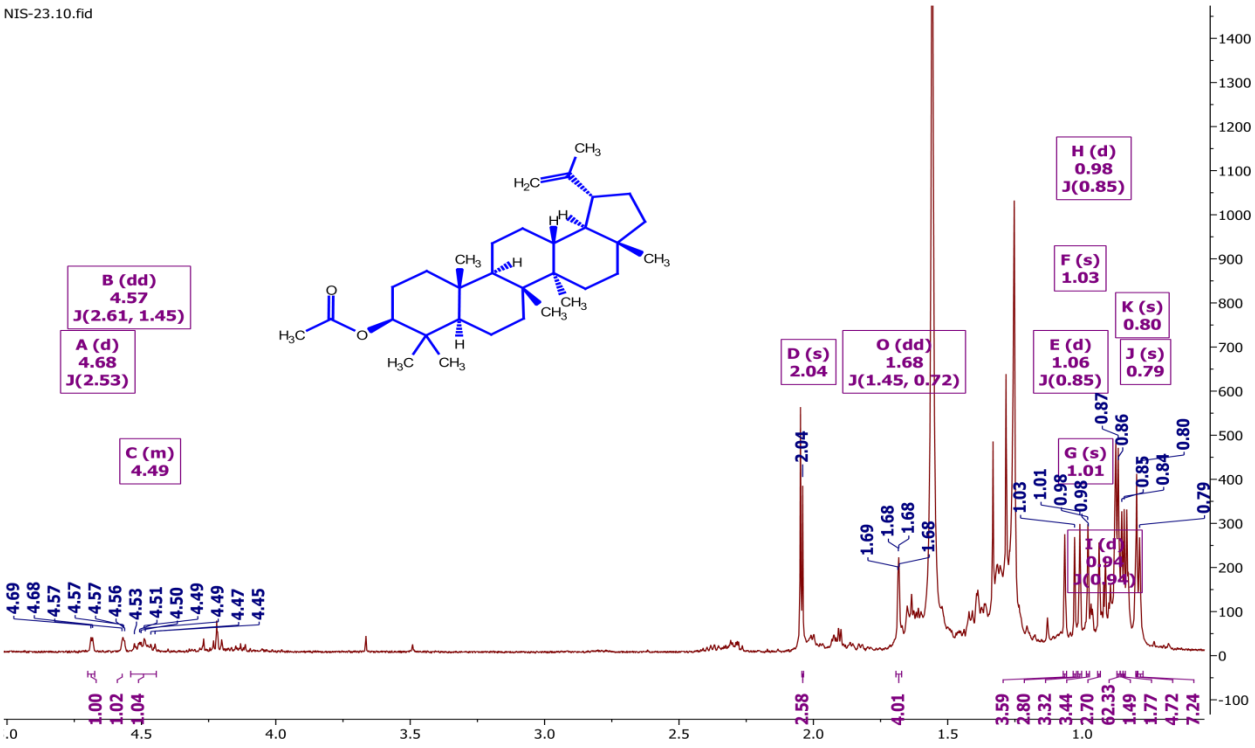


FIG 2: H-NMR SPECTRA OF LUPEOL ACETATE

Conclusion

This study has shown that *Napoleona imperialis* seed contains varieties of pharmacological qualities, including the ability to treat wounds and have anti-inflammatory, antioxidant, anti-diarrheal, anti-hypertensive, anti-plasmodial, and hepatoprotective actions. It is also used to aid in postnatal recovery. Various parts of *Napoleona imperialis* are used by traditional healers to treat a range of ailments, such as sore throats, wounds, arthritis pain, colds, coughs, nausea, diarrhea, malaria, toothaches, and earaches. These features are due to the bioactive substances contained in the plant, stigmasterol, sitosterol, and lupeol acetate. This is due to the fact that they demonstrate a wide range of biological and pharmaceutical qualities, such as anticancer, anti-osteoarthritis, anti-inflammatory, anti-diabetic, immunomodulatory, anti-parasitic, antifungal, antibacterial, antioxidant, and neuroprotective capabilities.

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